



Citation article: Horditsa, V., Grynychuk, F., & Besaha, R. The determining the photoluminescence intensity for assessing of the small bowel viability in the experiment. *Futur Med [Internet]*. 2023;2(3):20-28. Available from: <https://doi.org/10.57125/FEM.2023.09.30.03>

The determining the photoluminescence intensity for assessing of the small bowel viability in the experiment

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Abstract

Aims: Explore the potential of utilising photoluminescence intensity (PhLI) indicators for evaluating bowel viability.

Methodology: PhLI of the small bowel walls on the initial, middle, and distal parts were measured in the control group (10 rats). The loop of the initial (10 rats), middle (10 rats) and distal (10 rats) part of the bowel with the mesentery was ligated. PhLI of the bowel walls were measured on the afferent (AP), efferent (EP) and ligated (LP) parts in 6 hours. LGN-503 argon laser with a wavelength of 458 nm and a power of 200 mW was used. Sections of bowels were taken for histological examinations.

Results: In AP revealed dystrophic disorders, in EP revealed minor changes, in LP revealed necrosis. The small bowel wall PhLI with altered viability decreased at the wavelength range $\lambda = 470-490$ nm, but the absolute indicators were significantly variable. The ratio of PhLI at wavelengths $\lambda = 474$ nm/ $\lambda = 489$ nm on the initial, middle, distal bowel's parts didn't significantly differ in control. The ratio on each bowel part didn't significantly differ on the AP, EP, LP. The ratio's differences of the viable and non-viable bowels were significant on each part.

Conclusion: 1. The small bowel wall PhLI with altered viability decreases at the wavelength range $\lambda = 470-490$ nm. 2. The ratio of the small bowel walls PhLI indicators at the wavelengths $\lambda = 474$ nm/ $\lambda = 489$ nm is statistically significantly different in the bowel parts without altered viability and the bowel parts with necrosis. 3. Indicators of the ratio > 17 units testify the absence of viability altered, indicators in the range of 13-17 units testify the morphological disorders without necrosis, indicators < 13 units testify the bowel necrosis. 4. The determination of this ratio is promising for use in patients to assess its informativeness.

Keywords: Abdominal Surgery; Small Bowel; Viability; Laser Ray; Luminescence spectra.

Received: July 7, 2023

Revised: July 20, 2023

Accepted: September 1, 2023

Published: September 30, 2023

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DOI: <https://doi.org/10.57125/FEM.2023.09.30.03>

Introduction

Assessing of the bowel viability is one of the main issues during bowel obstruction surgery [1-4]. Consequences of assessing viability errors are serious complications. These complications can lead to serious health consequences,

requiring emergency surgery and potentially resulting in sepsis, organ failure, and even death. The results of suturing or anastomosing a non-viable bowel are suture leakage and peritonitis. On the other hand, the result of misdiagnosing the bowel as non-viable is bowel over-resection, bowel shortening, short bowel syndrome and digestive disorders.

Various ways have been proposed for the viability assessment [6-15]. Most of these ways determine the state of blood circulation in the vessels of bowel walls. For this, dyes are injected into the blood vessels. But the injection of a dye into a bowel vessel has a number of imperative disadvantages. First of all, the dye spreads through the mesenteric vessels, vessels of the muscle layer, submucosal layer. During the examination, a colored area of the bowel is visible, but it is not clear whether the dye has spread into the mucosal layer of the bowel. At the same time, it is known that necrosis begins precisely from the mucous layer. In addition, the vessels of the mucous layer may be damaged and the dye will spread through the damaged vessels into the tissue. The outcome is the discoloration of the tissues and the incorrect classification of the bowel as viable. The reason for this could be the lack of dye spreading caused by vasospasm. This leads to the misdiagnosis of the bowel as non-viable.

During urgent operations, the possibilities of using this way are limited. Most diseases that require urgent surgery cause swelling of intestinal tissues, mesentery, anatomical disorders (torsions, deformations, adhesions, etc.). Therefore, the vessels visualisation is problematic. Eventually, the injection into a vessel can disrupt the blood circulation and the bowel viability. As a result, intestinal necrosis can occur after surgery with negative consequences.

Therefore, the ways that do not use injections and evaluate the condition of all bowel tissues, and not only the bowel vessels, are more effective [14, 16, 17]. However, the well-known ways are not widely used. Technical complexity, low informativeness, etc. are the reasons for this.

Particularly, the way of electrical impedance spectroscopy [14] allows determining the impedance on the bowel surface. In addition, the authors note that the impedance decreased, then increased the next two hours, before decreasing until the end of the experiment. At the same time, research was conducted on the resected parts of the intestines. That is, those parts were already non-viable.

The laparoscopic laser speckle contrast imaging way [16], in fact, determines the state of microcirculation. The disadvantages of this way are described above.

The tissue oxygen measurements way determines the tissue oxygen saturation [17]. But the tissue saturation is a very variable parameter. The normal parameters of this criterion are unknown. Mean StO_2 of normally perfused colon was 79.4% while mean StO_2 of the small bowel was 60.5% which was statistically significant ($P < 0.0001$). Therefore, it is not clear what can be considered normal parameters. In addition, elevated StO_2 , according to the authors, was even to the devascularised segment of colon. Therefore, further search for effective viability assessment ways is needed. The way of bowel viability assessing must meet certain requirements. During the application of the way, invasive procedures should not be applied. A subjective evaluation of a conditionally viable bowel should not be used to assess viability. The assessment should be comprehensively carried out for all layers of the bowel wall. The assessment should take into account different criteria of bowel wall viability.

Research Problem

The research problem, which was the basis of this study, consisted in evaluating the informativeness of using a double parameter of the intensity of the small bowel walls photoluminescence to assessment its viability.

Research Focus

This study aimed to experimentally compare the photoluminescence intensity of small bowel walls with and without viability deviations. The objective was to assess whether the small bowel viability could be determined by examining the indicators of photoluminescence intensity in the walls.

Research Aim and Research Questions

1. To evaluate the indicators of the small bowel walls photoluminescence intensity without viability deviations and with viability deviations.
2. To study the differences between the indicators of the small bowel walls photoluminescence intensity.
3. To investigate the possibility of using indicators of the small bowel walls photoluminescence intensity to assessment the small bowel viability.

Research Methodology

General Background

The viability of the bowel can be accurately determined through histological examinations, which involve studying the tissue sample under a microscope. To validate and cross-check the accuracy of these examinations, photoluminescence intensity measurement data can be compared with the histological findings.

In order to carry out this investigation, a controlled experiment should be conducted.

Sample / Participants / Group

40 intact white non pedigree female rats. All rats were sexually mature (age 6 months). The weight of the rats was 180-200 g. Before the start of the experiment, the rats were in a vivarium. The conditions of stay and food were the same for all rats. 10 intact rats were the control group. 30 animals were the experimental group. A laparotomy was performed on the rats. The luminescence spectra of the walls of the initial, middle, and distal parts of the small bowel were measured in the control group. The loops of the initial (10 rats), middle (10 rats) and distal (10 rats) parts of the small bowel with the mesentery was ligated in the experimental group. During the experiment, the rats were in the same conditions and received the same drink.

The criteria for selecting rats for the control group and the experimental group were the same age, weight of 180-200 g, absence of diseases in rats. Rats for the control group were randomly selected from the 40 rats taken for the experiment.

The control group and the experimental group were homogeneous in terms of age, sex, animal weight, and living conditions. The average weight of rats in the control group was 191.5 ± 2.21 . The average weight of rats in the experimental group was 190.76 ± 1.19 ($p > 0.05$). Therefore, the control group and the experimental group were comparable. This made it possible to obtain comparative data and make reliable conclusions.

The control group was used to obtain data from intact animals. Such data are needed for comparison with the data obtained during the experiment.

Instrument and Procedures

The luminescence spectra of the small bowel walls were measured in the afferent (AP), efferent (EP) and ligated (LP) parts in 6 hours. Sections of bowels were taken for histological examinations after measuring the luminescence spectra.

The bowel walls were irradiated with a monochromatic laser ray. Its source was an LGN-503 argon laser with a wavelength of 458 nm and a power of 200 mW. The laser was powered by an AC 220 W 50 Hz electrical network. The laser ray was filtered by the FS-1 filter, which eliminated radiation with a wavelength of $\lambda > 460$ nm and created an irradiation of 6×10^{-6} W/m² of the bowel. The laser radiation scattered by the bowel wall was focused on the input slit of the MDR-12 monochromator, behind which the ZhS-16 light filter was mounted. At the out from the monochromator, the laser ray fell on a photodetector connected to a universal voltmeter V-7-21A, which was used to determine the output parameters of radiation (fig. 1). The photodetector was powered by an AC 220 W 50 Hz electrical network. The temperature lamp TRSh 2850-3000 was used as a reference radiation source for decoding the luminescence spectrum.

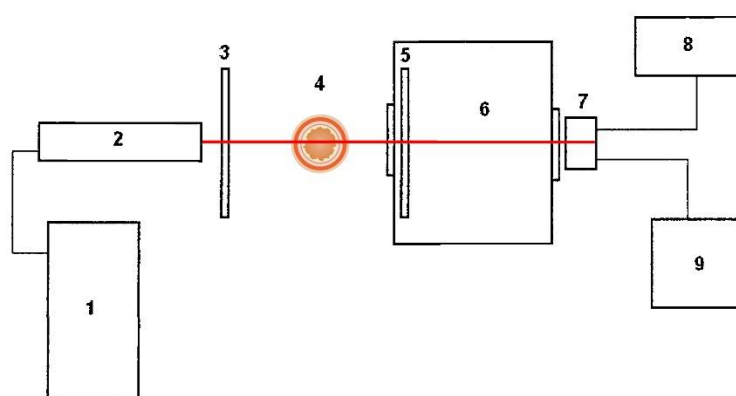


Fig. 1. Scheme of the experimental setup:

1 - laser's power unit; 2 - argon laser; 3 - light-filter; 4 - bowel; 5 - light-filter; 6 - monochromator; 7 - photodetector; 8 - universal voltmeter; 9 - photodetector power unit.

Bowel tissues were fixed in the 10% Neutral Buffered Formalin solution. Fixation in histology refers to the application of chemicals to protect the cellular structure from deterioration and preserve the organic tissue structure. When doing the investigation under a light microscope, neutral buffered formalin is typically used. Fixatives improve tissue and cell preservation by cross-linking proteins in an irreversible manner. The fixation phase preserves the chemical makeup of the tissues, makes cells or tissues more rigid for cutting, and postpones deterioration. The Neutral Buffered Formalin provides good tissue and cell structure preservation while stabilizing amino acids in proteins. Bowel tissues were dehydrated in an ascending alcohol battery. The goal of this phase was to remove water from the chosen tissues in order to harden them and make it easier to cut them into thin sections for use in light

microscopes. Then bowel tissues were embedded in paraffin wax for histological examination. Embedding is accomplished using paraffin wax to make it simpler to extract cellular components. Paraffin wax is used to achieve good morphology in complicated biological tissues. After cooling, the tissue hardens, and was used to cut slices (sectioned). Sections were made on a microtome with a thickness of 5 μm . The sections were passed through xylene, and then decreasing strengths of alcohol (100% to 0%) and finally water. Deparaffinized sections were stained with hematoxylin-eosin.

The following technique of staining the sections was used: Hematoxylin (3 minutes), Water wash (1 minute), Differentiator (1 minute), Water wash (1 minute), Bluing (1 minute), Water wash (1 minute), 95% ethanol (1 minute), Eosin (45 seconds), 95% ethanol (1 minute), 100% ethanol (1 minute), 100% ethanol (1 minute), Xylene (2 minutes), Xylene (2 minutes), Coverslip [18].

Hematoxylin and eosin stains have been used for at least a century and are still essential for recognising various tissue types and the morphologic changes that form the basis of diagnostics of morphological disorders in tissues, diagnostics of tissue viability. The stain discloses abundant structural information, with specific functional implications [18].

Stained preparations were studied in a Delta Optical Evolution Pro 100 light microscope. The state of the bowel wall different layers, the blood vessels state, and the cells state were studied. The results of studies of intestines in intact animals were compared with the results of studies of different sections of intestines in experimental animals and known scientific data regarding the condition of bowel tissues and signs of damage to bowel tissues for the interpretation of the results of histological research.

Inhalational sevoflurane anaesthesia was used for analgesia. Animals were removed from the experiment by an overdose of anaesthetic.

While performing the work, the norms of conducting research in the field of biology and medicine were observed: the Vancouver Conventions on Biomedical Research (1979, 1994), the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (1986).

Data Analysis

Testing of the law of distribution of samples for normality was carried out using the Shapiro-Wilk test. Shapiro-Wilk test has the best power for a given significance [19]. This test is used for small sample sizes. The null-hypothesis of the Shapiro-Wilk test is that the population is normally distributed. Thus, if the p value is less than the chosen alpha level, then the null hypothesis is rejected and there is evidence that the data tested are not normally distributed. On the other hand, if the p value is greater than the chosen alpha level, then the null hypothesis (that the data came from a normally distributed population) cannot be rejected.

To test the hypothesis of equality of means, the Wilcoxon test was used, since the data distribution in the samples differed from normal. The Wilcoxon signed-rank test is a non-parametric statistical hypothesis test used either to test the location of a population based on a sample of data, or to compare the locations of two populations using two matched samples. For two matched samples, it is a paired difference.

The null hypothesis assumed that there was no significant difference between the data that were compared. The alternate hypothesis supposed that there was a significant difference. If the p-value was greater than alpha, then the null hypothesis can't be rejected and it was assumed that there was no significant difference between the two samples. One of the most common ways to measure the similarity of two sets was to compare their data summary via mean and median.

Alpha is also known as the level of significance. This represents the probability of obtaining your results due to chance. The smaller this value is, the more "unusual" the results, indicating that the sample is from a different population than it's being compared to, for example. Commonly, this value is set to .05 (or 5%). We have set the level of significance 0.05.

Research Results

Histological examinations in the control showed no changes in the bowel structure. Histological examinations in the AP of the bowels revealed morphological disorders without signs of necrosis (dystrophy, oedema). Histological examinations in the EP of the bowels revealed minor changes (fullness of venous vessels). Histological examinations in the LP of the bowels revealed necrosis. The results of measuring the small bowel photoluminescence intensity of intact animals are shown in figure 2.

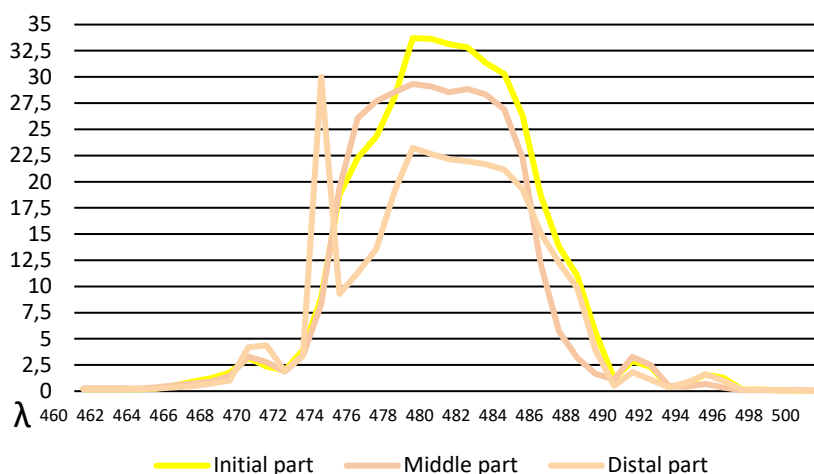


Fig. 2. The small bowel photoluminescence intensity in intact animals

The results of measuring the small bowel photoluminescence intensity of the small bowel necrotised parts (LP) are shown in figure 3.

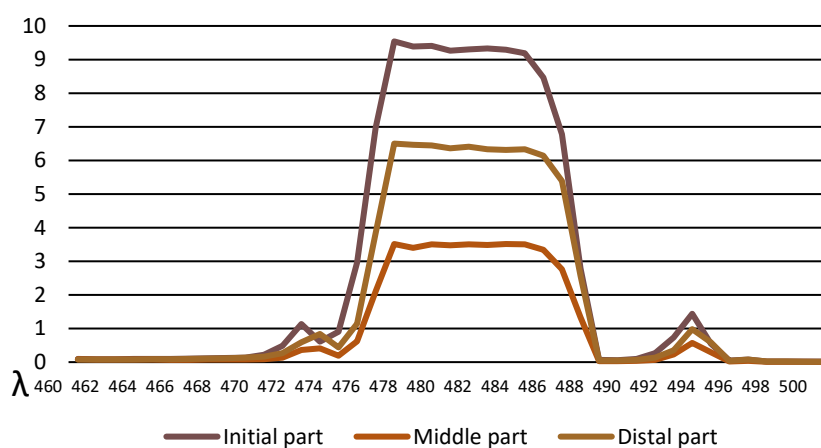


Fig. 3. Photoluminescence intensity of the small bowel necrotised parts

The ratio of photoluminescence intensity indicators at different wavelengths in the walls of the bowel intact parts and non-viable bowel parts were estimated. We found that the ratio of indicators determined at wavelengths $\lambda = 474$ nm and $\lambda = 489$ nm was informative. The values of the indicators of the ratio $\lambda = 474/489$ nm are shown in figure 4.

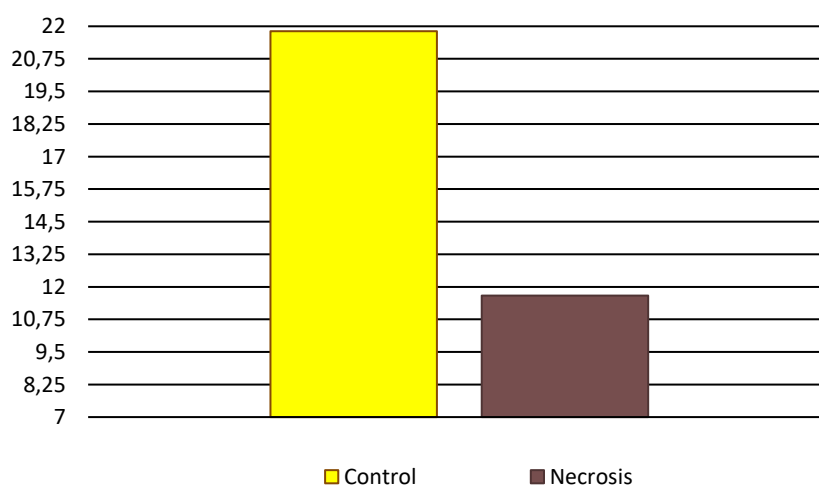


Fig. 4. Indicators of the photoluminescence intensity ratio at wavelengths $\lambda = 474 / \lambda = 489$ nm

Discussion

Comparing the results of measuring the photoluminescence intensity with the histological examinations data shows that due to the development of the bowel wall necrosis, indicators of the its photoluminescence intensity in the wavelength range $\lambda = 470-490$ nm decrease. This is a consequence of different processes: impaired blood circulation, oedema, tissue destruction. The tissues of the intact bowel are well filled with blood. The blood moves through the vessels, evenly filling the tissues. Therefore, these tissues luminesce more strongly [20]. Due to the destruction of the bowel walls tissues, their blood supply decreases. At the same time, blood circulation slows down in viable tissues. Therefore, blood stagnation occurs in these tissues. The blood becomes thicker. If the blood has penetrated the tissues through damaged vessels, this blood clots. Therefore, the bowel tissues photoluminescence intensity decreases. But the determination of the photoluminescence intensity evaluates total blood circulation disorders, not only the blood filling of vessels, as some known methods [10-12,15]. This happens because the laser ray penetrates through the tissues of the bowel wall. The laser ray causes luminescence of all bowel wall layers. Therefore, the photoluminescence intensity parameters show the total changes in the state of the tissues. So, a decrease in the photoluminescence intensity more accurately describes the violation of the bowel walls viability.

At the same time, absolute indicators of photoluminescence intensity are highly variable. The results of the study showed that these indicators significantly differed in different parts of the bowel (fig. 1). Numerous factors affect the photoluminescence intensity indicators. Among such factors are individual differences, which are evidenced by the data distribution, which differs from the normal according to the Shapiro-Wilk test. In addition, these are local features, the degree of bowel filling.

The animals of the control and experimental groups were similar in basic characteristics. But the variability of the photoluminescence intensity indicators was affected by a number of factors. First of all, the method of measuring the intensity of photoluminescence is very sensitive. Indicators of photoluminescence intensity were affected by the smallest factors [20].

The rats were in the vivarium before the start of the experiment. The rats had free access to food and water. Therefore, different animals could consume different volume of food and drink. This affected the degree of intestinal filling and, accordingly, the photoluminescence intensity indicators. It was the same in the rats of the research group. In addition, the animals of the experimental group had free access to drink after bowel obstruction modelling and, accordingly, could consume different volume of water. Different digestion processes take place in different bowel parts. Therefore, the bowel contents, the blood filling degree, the muscle contraction activity, etc., differ in different bowel parts. Therefore, these factors affect the differences in the photoluminescence intensity in the initial, middle and distal bowel parts. Each animal reacted individually to a stressful situation (experiment).

Bowel ligation in the research group was performed by one researcher. But the ligation was done manually. It was impossible to make the degree of bowel compression absolutely the same in each animal. Therefore, the degree of bowel compression in each animal could have been slightly different. The consequence of some differences in the degree of bowel compression could be some differences in pathological disorders in the bowel walls and, accordingly, the photoluminescence intensity indicators.

However, it should be noted that such variability is actually positive. In real clinical circumstances, patients differ much more than animals in an experiment, which cannot be taken into account in any way. Therefore, the specified individual characteristics somewhat display the variability of clinical conditions.

Of course, there are species differences – the indicators will differ in different animals and people, because the bowel histological structure is different. Therefore, it is advisable using relative indicators, which can neutralise the effect of the mentioned factors.

That is why the ratio of photoluminescence intensity indicators at wavelengths $\lambda=474/\lambda=489$ nm was used. As the research showed, in the control, the ratio in the initial part of the small bowel was 22.53 ± 1.44 , in the middle part – 19.09 ± 1.89 , in the distal part – 23.58 ± 1.98 units. There were no significant differences between indicators ($p > 0.05$). In the non-viable bowel, the ratio indicators in the initial part of the small bowel were 11.58 ± 3.26 , in the middle part – 7.40 ± 0.66 , in the distal part – 8.08 ± 0.62 units. There were no significant differences between indicators ($p > 0.05$).

The differences of the ratio of the viable bowel photoluminescence intensity (control, EP) and the non-viable bowel (LP) were significant in the initial part ($p < 0.01$), in the middle part ($p < 0.05$), and in the distal part ($p < 0.01$). The average indicators of the ratio of the viable bowel photoluminescence intensity (21.81 ± 1.05) and the non-viable bowel (11.66 ± 1.63) also differed significantly ($p < 0.01$). Indicators of the ratio of the dystrophically altered the bowel (AP) photoluminescence intensity were within 13-17 units.

Therefore, the study showed that the determination of the ratio of the small bowel walls photoluminescence intensity indicators at the wavelengths $\lambda=474/\lambda=489$ nm was promising for assessing the small bowel viability.

This way of the viability assessment has a number of significant advantages over known ways. First of all, it is the accuracy of viability assessment. The reasons for the increased accuracy are the features of this way. The laser ray

causes luminescence of all the bowel wall layers. Therefore, the photoluminescence intensity parameters show the total state of all the bowel wall.

In order to use this way, it is not necessary to carry out invasive manipulations: injections into vessels, which can disrupt the blood circulation and cause a disruption of bowel viability after the end of the surgery. To practice the way, it is not necessary to use additional substances that may cause allergic reactions.

The way peculiarity is also that viability can be determined on a limited bowel part. This is of significant importance in certain clinical situations, for example, if the question of bowel resection is being resolved in difficult anatomical zones: the initial part of the jejunum, the terminal part of the ileum, etc.

Objective assessment of the bowel viability is extremely important. The use of a reliable, objective way of viability assessment would avoid clinical errors. This would reduce the number of complications after surgery, reduce management costs, and improve the patient's quality of life.

However, it should be noted that currently this method cannot be used in patients. It is necessary to conduct additional experimental studies. It is also necessary to create a compact device for clinical use, but this is a task for engineers.

Limitations of the Study

The presented study has several limitations that should be addressed in future research. Firstly, the study used a small sample size of only 40 rats, although the total number of measurements in different parts of bowel walls was higher. Therefore, it is crucial to replicate the study using a larger sample size to ensure the reliability of the data.

Secondly, the previous study exclusively utilized female rats. To validate the findings, it is essential to include male rats in future studies. This will allow for a more comprehensive understanding of gender differences and potential variations in the results. Additionally, it is recommended to conduct experiments with different species of animals to examine the generalizability of the findings. The use of multiple animal models will strengthen the validity and applicability of the research. Moreover, experimental studies focusing on the large bowel should be conducted to expand our understanding of the topic. The inclusion of different segments of the bowel will provide a more comprehensive understanding of the subject matter. Although the limitations mentioned above need to be addressed, it is important to note that the known physiological and pathological regularities common to all warm-blooded mammals can minimize their impact on the generalizability of the findings. Consequently, it is anticipated that the fundamental regularities will be confirmed, albeit with potential minor variations in certain indicators. Ultimately, in order to definitively determine the validity of the experimental data, it is imperative to test the research findings in human subjects. This step will provide valuable insights into the potential translational applications and implications of the study results.

Prospects for Future Research

The presented research is part of the scientific work of the Department of Surgery № 1, Bukovinian State Medical University. One of the scientific work objectives is the complications prevention in abdominal surgery. The presented research is one of the scientific work series on ways to assessment the bowel viability. The results of other research will be shown in other publications. The aim of the research series is to find the optimal way to assessment the bowel viability. Research with a larger number of animals will be done in the future. Research will also be applied to patients.

Conclusions and Implications

1. The intensity of photoluminescence in the small bowel wall decreases at a wavelength range of $\lambda = 470-490$ nm when viability is altered.
2. The ratio between the indicators of photoluminescence intensity in the small bowel wall at wavelengths $\lambda=474/\lambda=489$ nm is significantly different between bowel parts without altered viability and bowel parts with necrosis.
3. Ratios greater than 17 units indicate the absence of viability alteration, ratios between 13-17 units indicate morphological disorders without necrosis, and ratios less than 13 units indicate bowel necrosis.
4. This ratio shows promise for assessing informativeness in patients.
5. Objective assessment of bowel viability in surgical practice will help improve the diagnosis of bowel necrosis, a common complication of bowel obstruction.

6. Improving the diagnosis of bowel necrosis will optimize the management of patients with bowel obstruction by selecting the best surgical intervention, reducing complications, lowering treatment costs, and enhancing the patient's quality of life.

7. The findings of this research are valuable to the medical community, as they provide guidance for improving management strategies for patients with various bowel disorders.

Declarations

Author Contributions

The following statements should be used:

Conceptualization, F. Grynchuk; methodology, F. Grynchuk and R. Besaha; validation, F. Grynchuk and R. Besaha; formal analysis, F. Grynchuk and R. Besaha; investigation, V. Horditsa; resources, V. Horditsa; data curation, V. Horditsa; writing-original draft preparation, V. Horditsa; writing-review and editing, V. Horditsa, F. Grynchuk; visualization, V. Horditsa; supervision, F. Grynchuk; project administration, F. Grynchuk. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Below are suggested Data Availability Statements.

- Data sharing not applicable: No new data were created or analysed in this study. Data sharing is not applicable to this article.

Funding

Funding information is not available.

Acknowledgements

Institutional Review Board Statement

While performing the work, the norms of conducting research in the field of biology and medicine were observed: the Vancouver Conventions on Biomedical Research (1979, 1994), the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes (1986).

Informed Consent Statement

Not Applicable.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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